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Analysis of surfactant leaf damage using microscopy and its relation to glyphosate or deuterium oxide uptake in velvetleaf (*Abutilon theophrasti*)

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Abstract: Commercial formulations of glyphosate were compared for surfactant leaf damage and glyphosate uptake. The formulations (Roundup® Ultra, Roundup®, and Touchdown® were diluted with water to 12.5 g AI litre^{-1} and applied as 1-μl drops to the first leaf adaxial surface. Tissues at application sites were examined by light, fluorescence and scanning electron microscopy. At 24 h after treatment, tissue necrosis was clearly visible with Ultra and Roundup®, but not with Touchdown®. The application sites of Ultra and Roundup®, but not with Touchdown®. The application sites of Ultra and Roundup® demonstrated a well-demarcated zone of injury showing extensive rupturing of cell membranes in both epidermal and mesophyll cells. Studies using blank formulations without glyphosate confirmed that tissue damage was caused by the surfactant formulants. Diluted formulations (12.5 g AI litre^{-1}) spiked with a minimum of [^{14}C-glyphosate were applied similarly. Time-course studies showed the fastest uptake with Ultra, followed by Roundup® and Touchdown®. Mobilization of glyphosate away from the treated leaf was proportional to uptake. The use of a deuterium NMR method demonstrated that pretreatment of leaves with glyphosate formulations facilitated subsequent leaf loading of deuterium oxide. The extent of the latter correlated with leaf loading of glyphosate in formulations. These results indicate that the role of the surfactant is to overcome the leaf cuticle and membrane barriers to facilitate glyphosate entry into the leaf.

Keywords: glyphosate; surfactant; velvetleaf; uptake; transport; deuterium oxide; NMR.

Glyphosate efficacy is directly related to its ability to penetrate the leaf surface. Surfactants are used routinely in herbicide formulations to improve leaf coverage, wetting and uptake. Glyphosate efficacy, in particular, is strongly influenced by surfactants.[1,2] The delivery efficiency of glyphosate was compared in three commercial formulations. Roundup® Ultra is Monsanto Company's newest formulation in the USA, replacing the previous Roundup® formulation; both formulations contain glyphosate isopropylamine salt in a cationic surfactant. Touchdown® (Zeneca's Canadian formulation) contains the glyphosate trimesium salt in an alkylpolyglucoside surfactant. All of these formulations contain equal concentrations of AI with similar prescribed use rates for weed control.

The microscopy studies examined the tissues at the sites of application of Ultra, Roundup, and Touchdown to leaves of velvet-leaf (*Abutilon theophrasti* (L) Medic). The adaxial surface of the first leaf (3.5–4-leaf plant) was treated with 1-μl drops of the formulations diluted to 12.5 g AI litre^{-1}. The leaves were excised at 24 hours after treatment (HAT), washed with water, and examined for tissue injury. Ultra and Roundup produced visible necrosis, which appeared as bright refractive patches on the leaf surface by epi-illumination stereo microscopy. In contrast, the Touchdown application sites displayed a dark footprint, but remained largely injury-free. The injured sites of Ultra and Roundup also displayed fluorescence when viewed with a fluorescein filter set in the absence of an added fluorochrome dye. Analysis by scanning electron microscopy of the tissue area treated with Roundup clearly showed a well-demarcated and irregularly shaped pit on the leaf surface. Tissue cross-sections of Roundup sites revealed collapsed upper and lower epidermal cell layers and a pycnotic mesenchymal layer.

A minimal amount of [^{14}C]glyphosate (<1% of glyphosate dose) was spiked into each formulation (12.5 g AI litre^{-1}) and applied as 1-μl drops to the adaxial surface of the first leaf (3–4-leaf plants). Uptake (Table 1), which was calculated by summing the radioactivity in the treated leaf and in the rest of the plant, was inversely proportional to that recovered from the treated-leaf wash. Total uptake was fastest with Ultra, reaching 35% of applied dose at 6 HAT and plateauing at 41% by 24 HAT. Roundup showed intermediate uptake followed by Touchdown. Although uptake in Touchdown was initially very low (5% at 6 HAT), it continued throughout the test period and had not reached a plateau by 72 HAT. The amount of radioactivity remaining in the

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(*Received 25 June 1998; accepted 25 November 1998*)

Hours after treatment (HAT)	[^{14}C]Glyphosate uptake[a] (% of applied close)(±SE)		
	Roundup Ultra	Roundup	Touchdown
3	18.6 (±1.4)	8.5 (±0.7)	2.8 (±0.5)
6	35.4 (±1.8)	21.9 (±0.9)	4.9 (±0.4)
24	40.7 (±2.3)	35.9 (±1.5)	13.9 (±1.3)
72	39.4 (±2.6)	32.3 (±1.1)	25.6 (±1.5)

Table 1. Total uptake of [^{14}C]glyphosate from commercial formulations when applied to velvetleaf

[a] Mean of six determinations.

treated leaf is a function of uptake minus the export into the plant. Radioactivity analysis of treated leaves showed rapid loading by Ultra, followed by Roundup and Touchdown.

The amount of glyphosate translocated away from the treatment site into the plant is believed to be a key indicator of efficacy. Glyphosate translocation was very fast with Ultra, followed closely by Roundup, with that from Touchdown being considerably slower. At 72 HAT, translocation of glyphosate with Ultra was more than twice that with Touchdown (19% vs 9% of applied dose). Although leaf loading of glyphosate from Ultra and Roundup reached a maximum by 6 HAT, the translocation of glyphosate did not reach a maximum until 24 HAT. This is evidence that the treated leaf represents an intermediary pool which serves as the source of glyphosate export into the phloem.

Using [^{2}H]NMR, we developed a simple method for measuring leaf permeability to water. Glyphosate is expected to be in close association with water, so that the impact of surfactants on leaf permeability to water should also translate to glyphosate. The objective was to determine whether deuterium oxide might be used as a marker for glyphosate in plant uptake studies. The experiment involved pretreating the leaf with diluted glyphosate formulation (12.5 g AI litre^{-1}) followed by application of deuterium oxide to the same drop sites. The control plant received the formulation and deuterium oxide at the same time. In order to reduce biological variability, diluted Roundup (12.5 g AI litre^{-1}), used as the internal control, was applied to one-half of the leaf and the second formulation (Ultra or Touchdown) was applied to the other half of the same leaf. Leaves were pre-treated with the formulations for 6 h, followed by a 30-min deuterium oxide treatment time. Relative to Roundup, Ultra and Touchdown showed 197% and 68% deuterium oxide leaf penetration, respectively. These results correlate with the uptake of [^{14}C-]glyphosate from these formulations.

The examination of three commercial formulations of glyphosate has identified distinct patterns of glyphosate uptake and translocation, and of leaf damage. We observed a direct correlation between the speed and the extent of uptake with leaf damage. Tissue damage within 24 h after application was caused by the surfactant, based on examination of blank formulations containing no glyphosate. Our results suggest that surfactant tissue damage plays a key role in penetration of glyphosate into the leaf.

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Ecdysone agonists – Mechanism of action and application on *Spodoptera* species

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Abstract: Laboratory assays using artificial diet demonstrated that tebufenozide (RH-5992) and the new structural analogue, RH-2485 (proposed common name methoxyfenozide), possess strong ecdysone-like activity against last-instar larvae of the beet armyworm, *Spodoptera exigua*, and the cotton leafworm, *Spodoptera littoralis*, leading to precocious lethal moulting. LC_{50} values showed that the activity of RH-2485 (0.38 mg AI litre^{-1}) was about twice that of tebufenozide (0.60 mg AI litre^{-1}) in *S. exigua*, whereas in *S. littoralis* respective LC_{50} values were 1.15 mg AI litre^{-1} and 9.51 mg AI litre^{-1}. The retention-fate curves of ^{14}C-radiolabelled ecdysone agonist could not explain the differential toxicity values between species and compounds. Ingestion of the oxidase inhibitor piperonyl butoxide (PB) synergized the toxicity of the ecdysone agonist, indicating the importance of oxidative detoxifcation in *Spodoptera* larvae, and may raise the possible use of PB as synergist for this group of insecticides, or for monitoring resistance due to increased oxidation.

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(*Received 8 July 1998; accepted 25 November 1998*)